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A Gas-Liquid Chromatographic Method for
the Determination of Permethrin in Oily Crops

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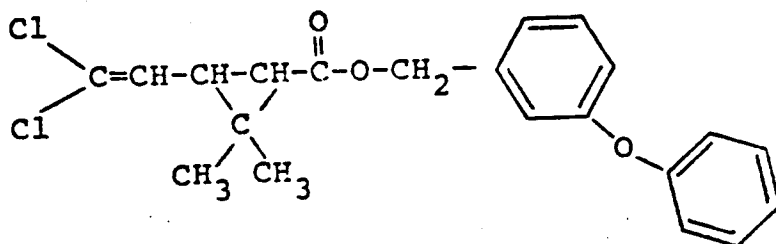
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A Gas-Liquid Chromatographic Method for the Determination of Permethrin in Oily Crops

1. Scope

The method is suitable for the quantitative determination residues of the *cis* and *trans* isomers of permethrin in oily crops such as cotton and corn. The limit of detection is 0.01 ppm of total permethrin.



Permethrin -- (3 Phenoxyphenyl) methyl (+)-*cis,trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Molecular weight: 391.28

2. Method Summary

Crop samples are extracted with 20% acetone in *n*-hexane, the extract poured through glass wool to remove insolubles and the solvent removed with a rotary evaporator. The residue is redissolved in 15% dichloromethane in cyclohexane and cleaned up by gel permeation chromatography. The solvent is again removed by rotary evaporation, the residue dissolved in *n*-hexane, and further cleanup done with a Florisil column. The solvent is removed by rotary evaporation, the residue redissolved in *n*-hexane, and the resulting solution analyzed by gas-liquid chromatography using an electron capture detector.

3. Reagents

- n*-hexane, pesticide quality
- Acetone, pesticide quality
- Cyclohexane, pesticide quality
- Dichloromethane, pesticide quality
- Diethyl Ether, pesticide quality
- Sodium sulfate, anhydrous, ACS
- Florisil, PR
- 5% OV-210 on 80/100 Gas Chrom Q
- Permethrin standards

4. Apparatus

- Wiley Mill or other suitable apparatus for grinding the samples.

- b. Sorval Omni-Mixer or other suitable high speed blender.
- c. Glass columns, 30 cm x 2.5 cm with Teflon stopcocks with a 60 ml reservoir at the top.
- d. Gas Chromatograph fitted with an electron capture detector capable of operating at 300°C.
- e. Automated preparative gel permeation chromatograph (Analytical Biochemistry Laboratories).

5. Procedure

5.1 Gas Liquid Chromatography

- a. Glass column 180 cm x 0.2 cm ID packed with 5% OV-210 on 80/100 Gas Chrom Q and conditioned at 270°C for 24 hours with 60 ml/min carrier gas flow.
- b. Temperatures: Column oven, 215°C; Injector, 240°C; Detector, 300°C.
- c. Carrier gas flow rate - 60 ml/min.

5.2 Gel Permeation Chromatography

- a. Column - 30 cm x 2.5 cm packed with 55 grams of Bio Beads Sx-3, 200 to 400 mesh.
- b. Solvent - 15% dichloromethane in cyclohexane.
- c. Pumping rate - 5 ml/min.
- d. Dump 130 ml, collect 60 ml, wash 10 ml.

5.3 Determination

- a. Thoroughly mix the chopped sample and weigh a representative aliquot containing approximately 2 g of lipid into a blender. Add 100 ml of 20% acetone in *n*-hexane and blend for 2 minutes.
- b. Filter the mixture through a glass wool plug in a funnel. Rise the blender with 2 - 50 ml portions of *n*-hexane and pour through the funnel. Collect the filtrate and washings in a 500 ml boiling flask.
- c. Evaporate to dryness on a rotary evaporator at a temperature not exceeding 40°C.
- d. Redissolve the residue in 20.0 ml of 15% dichloromethane in cyclohexane and transfer 10 ml to a 15 ml centrifuge tube.
- e. Centrifuge to remove insolubles.
- f. Inject 5.0 ml of the clear solution into the gel permeation chromatograph. Elute at the rate of 5.0 ml per minute and collect the 130 to 190 ml fraction in a 125 ml boiling flask.
- g. Evaporate to dryness on a rotary evaporator at a temperature not exceeding 40°C.
- h. Prepare a 5 gram (25 mm x 22 mm) Florisil column covered with about 10 mm of anhydrous sodium sulfate. This column should be dry packed.

- i. Transfer the residue from g to the Florisil column with 3-2 ml portions of n-hexane.
- j. Elute with 55 ml (this volume will vary with each batch of Florisil) of 10% diethyl ether in n-hexane.
- k. Evaporate to dryness on a rotary evaporator, redissolve the residue in n-hexane, and adjust the volume to exactly 5.0 ml (1.0 ml for oil samples).
- l. Calibrate the gas-liquid chromatograph with permethrin standard solutions.
- m. Analyze the sample solutions. Calculations can be done using peak height or peak area comparisons.

NOTE: When using GLC peak height comparison the apparent ratio of cis : trans will be high if it is assumed that the two isomers give equal weight responses. Because of this, peak area comparisons is the preferred method of calculation. Both methods give accurate total residue values.

6. Accuracy and Precision

Analytical standard permethrin was analyzed using the method described. The results in Table I show that the method gave quantitative recoveries of 50 to 1250 nanograms of added permethrin. The relative standard deviation was 18.1 for 50 ng of added permethrin and decreased with higher amounts to 5.7 for 1250 ng of added permethrin.

Similar results were obtained for permethrin added to ground ginned cottonseed. The results in Table II show that quantitative recoveries with good precision were obtained from concentrations of 0.02 to 0.50 ppm.

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Table I

Recovery of Permethrin from Standard Solutions

<u>Added, ng</u>	<u>No. of Analyses/a</u>	<u>Recovery</u>			
		<u>Mean, ng</u>	<u>Percent</u>	<u>Std. Dev.</u>	<u>Rel. Std. Dev.</u>
50	5	52.5	105	9.5	18.1
125	5	133.5	107	9.8	7.3
250	5	246.5	99	16.8	6.8
1250	5	1188.5	95	67.3	5.7

a/ each analysis of each concentration done on a separate day.

Table II

Recovery of Permethrin Added to Cottonseed

<u>Amount Added</u>		<u>No. of Analyses</u>	<u>Recovery</u>			
<u>ppm</u>	<u>ng</u>		<u>Mean, ng</u>	<u>Percent</u>	<u>Std. Dev.</u>	<u>Rel. Std. Dev.</u>
0.02	50	7	58.2	116	14.6	25.1
0.05	125	7	130.0	108	22.0	16.9
0.10	250	8	250.3	100	21.7	8.7
0.50	1250	10	1204.2	96	78.4	6.5

a/ each analysis of each concentration done on a separate day.

Permethrin in Cottonseed

0.07 ppm
trans

0.05 ppm
Cis

0.14 ppm
Trans

0.10 ppm
Cis

0.24 ppm
Trans

0.16 ppm
Cis

2.13

9/10/76

3-3

2.05

9/10/76

2-3

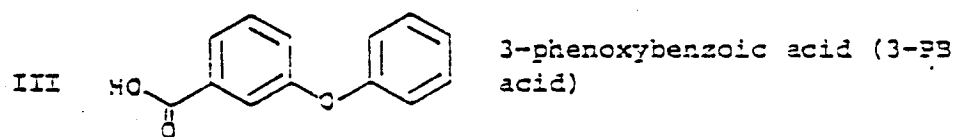
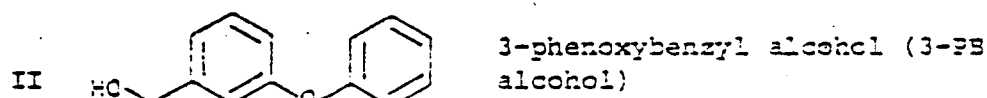
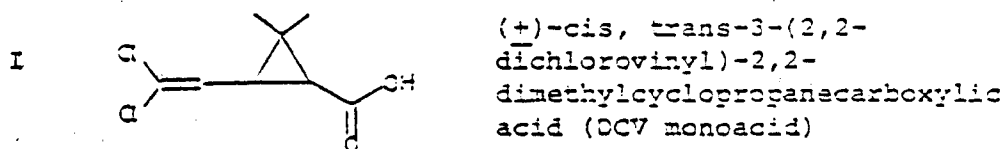
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APPENDIX I

MULTIRESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF METABOLITES OF PERMETERIN IN MILK AND ANIMAL TISSUES

1. SCOPE

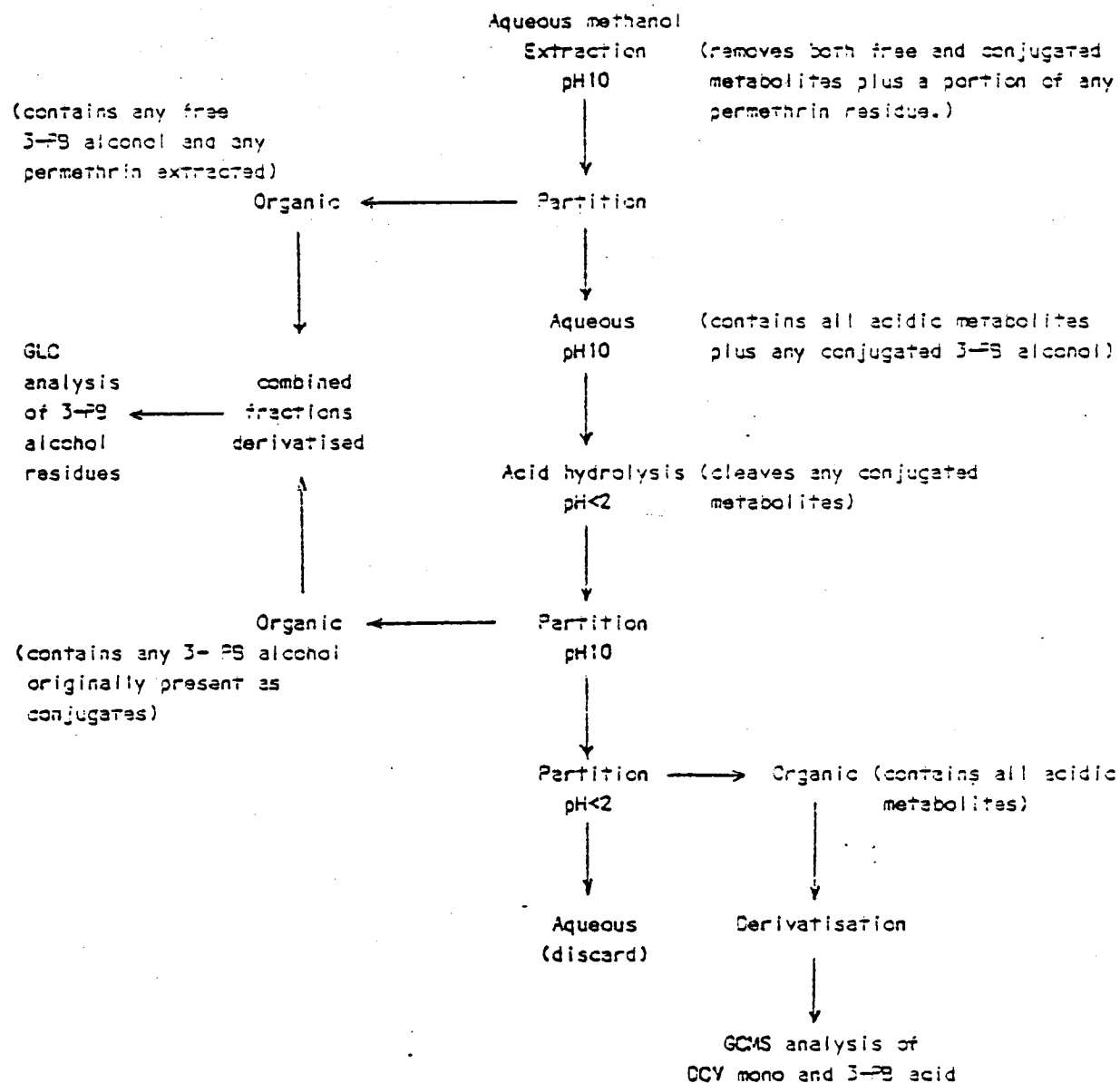
The method is suitable for the quantitative determination of metabolites I and II, III in milk and animal tissues. An acid hydrolysis step is included to allow cleavage of any conjugates of the metabolites.



The limit of determination of the method is 0.01 mg kg^{-1} of DCV monoacid, 3-PB alcohol and 3-PB acid.

Scheme 1

Steps used in the assay procedure for permethrin metabolites in cow tissue samples *.



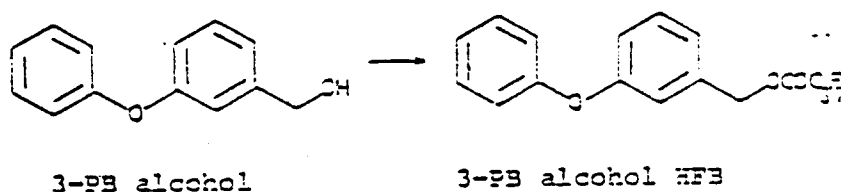
* Milk samples have the extraction and first partition combined into one step.

2.

SUMMARY

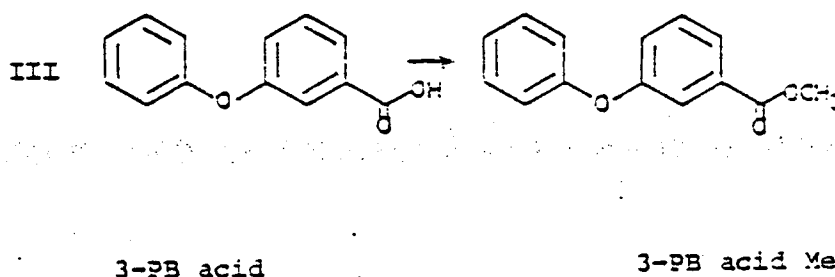
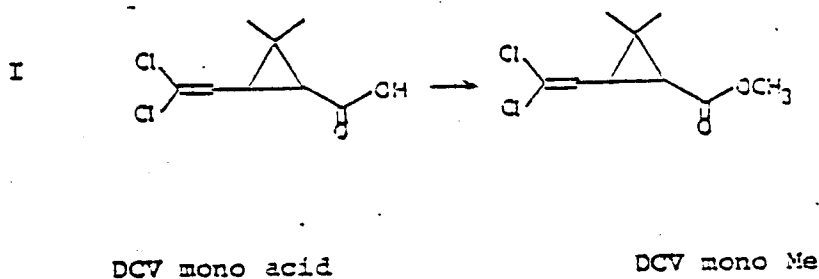
A schematic outline of the procedures used is shown in Scheme 1.

In summary, methanol:water is used to extract both free and conjugated residues of the metabolites. Any parent permethrin extracted by this procedure is partitioned out, along with residues of "free" 3-PB alcohol, into an organic solvent. The remaining acidic and conjugated residues in the aqueous phase are subjected to an acid hydrolysis step which cleaves any conjugated forms of the metabolites. 3-PB alcohol residues liberated in this way are combined with the initially separated "free" 3-PB alcohol and the combined residues treated with heptafluorobutyric anhydride (HFBA) to form the heptafluorobutyryl derivative of the alcohol.



Quantitative determination of the 3-PB alcohol HFB is then carried out by gas-liquid chromatography using electron capture detection.

The acidic metabolites I and III (of both free and conjugated origin) are collected in a single fraction after the hydrolysis step. The metabolites are converted to their methyl ester derivatives by treatment with diazomethane.



The methyl esters are quantitatively determined using gas chromatography - mass spectrometry (GCMS) operated in the selected ion monitoring (SIM) mode.

A modified procedure, in which the initial extraction and subsequent removal of parent permethrin and free 3-PB alcohol are combined in a single step, was used for milk samples.

3.

REAGENTS

(a) Organic solvents, redistilled from glass : chloroform, dichloromethane, diethyl ether, hexane, methanol and toluene. Available from Rathburn Chemical Ltd, Scotland, UK.

(b) Laboratory reagent chemicals, Analar grade : ammonium chloride, concentrated hydrochloric acid, sodium bicarbonate, sodium hydroxide, sodium sulphate (granular anhydrous). Available from BDH, Poole, UK.

(c) Derivatisation reagents, especially purified for electron capture gas chromatography; heptafluorobutyric anhydride, pyridine. Available from Pierce and Warriner, (UK) Ltd.

Diazomethane (ethereal solution) freshly prepared in the laboratory using a Diazaid[®] kit available from Aldrich Chemical Co. Inc.

(d) Glass wool, contaminants are removed by treatment with refluxing hexane in a Soxhlet apparatus for 2 hours.

(e) Celite 545 filter aid, washed with methanol prior to use. Available from BDH, UK.

(f) Whatmans No 41 filter papers.

(g) Fractosil SI 200 silica gel, available from BDH, UK.

(h) Stationary phase for gas-liquid chromatography, a phenyl-methyl silicone, OV17, coated onto a support material Chromosorb WEP (80 - 100 #). Available from chromatography suppliers eg Phasesep, UK, Ltd.

(i) Gas for GLC - 5% methane in argon (for EC-GLC) and helium (for GCMS), dried by passing through molecular sieve type 5A. Available from BCC, UK.

(j) Samples of I) dichlorovinyl monoacid
II) 3-Phenoxybenzyl alcohol
III) 3-Phenoxybenzoic acid

4.

APPARATUS

- (a) Equipment for the initial preparation of samples, e.g. Hobart food mincer.
- (b) High speed macerator, e.g. Sorval Omni-mixer.
- (c) Laboratory glassware: Mason jars (500ml), measuring cylinders (10ml - 250ml), pipettes (1ml - 10ml), Buchner flasks and funnels (500ml), round bottomed flasks (50ml - 250ml), glass stoppered centrifuge tubes (10ml), glass chromatography columns for:
 - (i) adsorption liquid chromatography (1cm I.D. x 25cm length)
 - (ii) for gas-liquid chromatography (180cm x 0.2cm I.D.)
- (d) Rotary evaporation apparatus, e.g. Buchi.
- (e) Manifold for providing gentle stream of dry air for evaporation of small volumes of solvent.
- (f) Extrelut^R partition columns available from Merck, Germany or suppliers, BCH, UK.
- (g) Ultrasonic bath e.g. MSE.
- (h) Gas-liquid chromatograph fitted with an electron capture detector, e.g. Hewlett Packard 5710 series fitted with Ni⁶³ (15mCi) model 1873 electron capture detector.
- (i) Syringes for gas-liquid chromatography, e.g. Hamilton 10ul

Note - the use of an autosampler apparatus with GLC equipment, e.g. Hewlett Packard 7671A, is satisfactory provided (a) suitably precise injections are achieved, i.e. reproducibility better than 5%, b) no cross-contamination from consecutive injections is observed, and (c) that no contamination arises in the final sample due to the autosampler vials or vial caps.

- (j) Potentiometric pen recorder (LMV), e.g. Perkin Elmer 56 or equivalent instrument.

Note - the use of an electronic integrator for measurement of peak areas, e.g. Hewlett Packard 3351A GC data system or Hewlett Packard 3380A reporting integrator can be used (in addition to the chromatographic trace of the pen recorder) provided that the analyst is satisfied that the area response given is both accurate and precise.

- (k) Gas chromatograph - mass spectrometer capable of operating in the Selected Ion Monitoring (SIM) mode, e.g.
- (i) Gas chromatograph - Hewlett Packard 5710A with heated injection port.
 - (ii) Interface - V. G. Micromass single stage glass jet separator.
 - (iii) Mass spectrometer - V. G. Micromass 16F single focussing magnetic sector instrument equipped with a 12 amp magnet supply unit and water cooled magnet.
 - (iv) Selected Ion Monitoring unit - V. G. Micromass digital 8 peak x 8 channel programmable unit having an integrated "mass lock" facility.
 - (v) Multi-pen recorder - Rikadenki model DB6.

Note - While all the reagents and apparatus may be individually checked for purity, it is necessary to analyse reagent blank samples, where the complete procedure has been carried out in the absence of sample. This will enable the analyst to verify whether the system produces final chromatograms which are free of interference at the retention times of the metabolite derivatives.

5. PROCEDURE

5.1 Gas-Liquid Chromatography (GLC) for the Determination of the Heptafluorobutyryl Derivative of 3-Phenoxybenzyl Alcohol

Column - 180 cm x 0.2 cm ID 3% OV17 on Chromosorb WHP (80-100#).

Oven temperature 150°C; Injector temperature 200°C; Detector temperature 350°C; Carrier gas flow rate 60 ml min⁻¹.

Using the above conditions the heptafluorobutyryl derivative of 3-phenoxybenzyl alcohol chromatographs as a single symmetrical peak of retention time 6.2 minutes. Sensitivity is such that 0.2 x 10⁻⁹ g of 3-phenoxybenzyl alcohol converted to the heptafluorobutyryl derivative gives approximately 75% full scale deflection when injected on-column, with electrometer attenuation set at x 32 and the potentiometric recorder range on 1mV.

5.2 Gas Chromatography - Mass Spectrometry (GCMS) Operated in the Selected Ion Monitoring (SIM) Mode for the Analysis of DCV mono acid and 3-Phenoxybenzoic acid as their respective Methyl Esters.

5.2.1 Gas Chromatography

Column 180 cm x 0.2 cm I.D. 3% OV17 on Chromosorb WHP (80 - 100#), carrier gas (helium) flow rate 30 ml min⁻¹.

(a) DCV monoacid (methyl ester).

- (i) total isomers : oven temperature 175°C ; injection port temperature 200°C , gives a single peak at retention time 2.0 mins.
- (ii) individual cis/trans isomers : oven temperature 130°C ; injection port temperature 150°C , gives two peaks - cis isomer retention time 5.0 mins, trans isomer retention time 5.8 mins.

(b) 3-Phenoxybenzoic acid (methyl ester) : oven temperature 200°C ; injection temperature 250°C , gives a single chromatographic peak at 3.0 minutes.

5.2.2 Mass Spectrometry

Electron impact mode; source pressure 1×10^{-6} Torr, source temperature 220°C , separator temperature 220°C , electron energy 70 eV, filament current 200uA (trap stabilised), accelerating voltage 4KV.

Resolution for SIM set at 500 (10% valley definition) with flat topped peaks.

The full mass spectra of the methyl esters of the three acidic metabolites are shown in FIG 1.

The Selected Ion Monitoring (SIM) unit is programmed to initially monitor the following m/z values in the spectrum of each individual compound.

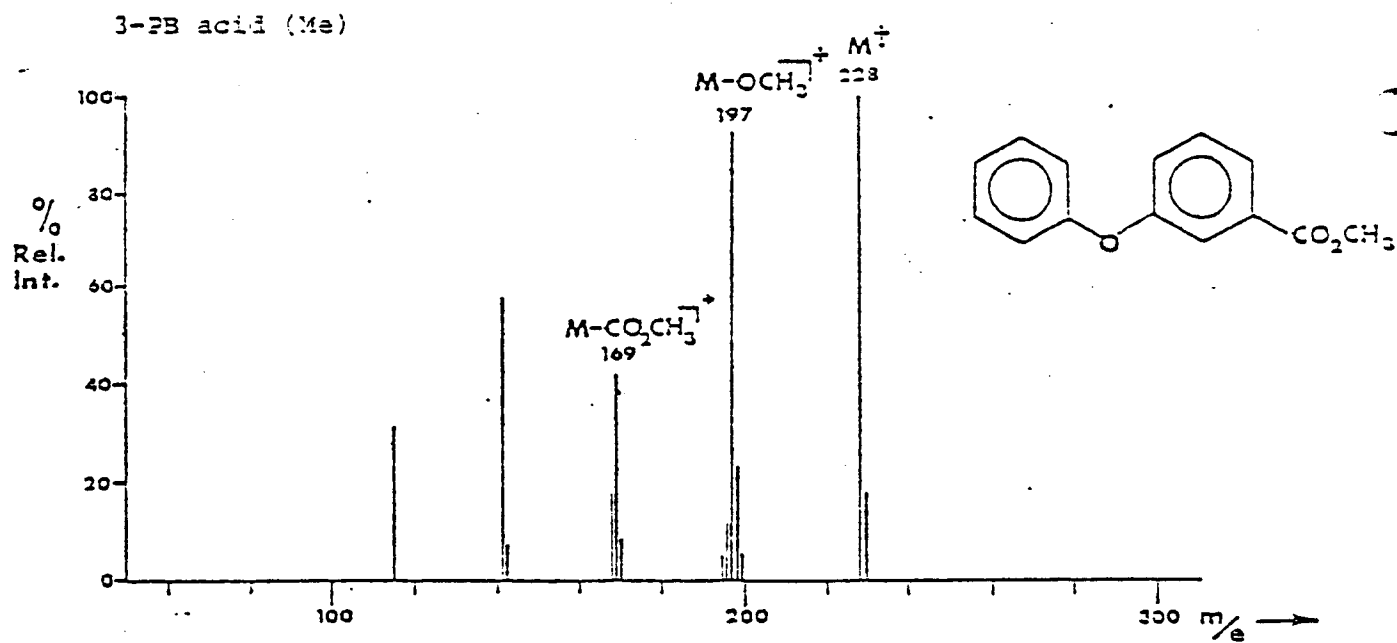
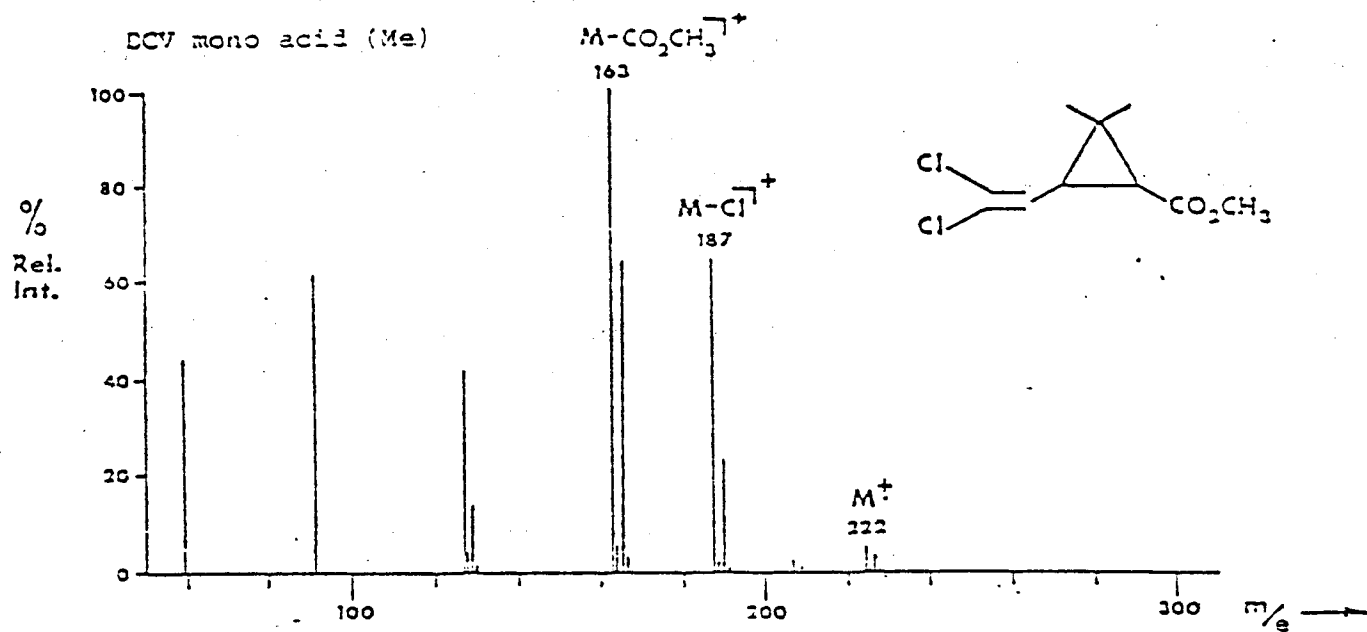
(a) DCV monoacid (methyl ester)

m/z monitored	ion species	% relative abundance
222	M^+	5%
187	$\text{M}-\text{Cl}^+$	67%
163	$\text{M}-\text{CO}_2\text{CH}_3^+$	100%

(b) 3-Phenoxybenzoic acid (methyl ester)

m/z monitored	ion species	% relative abundance
228	M^+	100%
197	$\text{M}-\text{OCH}_3^+$	90%
169	$\text{M}-\text{CO}_2\text{CH}_3^+$	42%

FIG 1 Electron impact (70eV) mass spectra of metabolite methyl esters



Note - For quantitative determination usually only one ion (that giving the highest signal noise ratio) is used for each metabolite.

5.3 Extraction of Metabolites and Partition of Parent Permethrin and Free 3-PB alcohol

5.3.1 Tissues

- (a) Weigh a representative sub sample (50 g) of the minced tissue into a 500 ml Mason jar. Add 50% v/v methanol : water adjusted to pH9 with sodium hydroxide solution (200 ml) and celite (10 g) and macerate at high speed for 5 minutes.
- (b) Filter the macerate through a Whatmans No 41 filter paper in a Buchner funnel. Wash the residuum with 50% methanol : water -pH10 (40 mls) and adjust the final volume of filtrate to exactly 250 mls using further MeOH: H₂O.
- (c) Transfer an aliquot (50 ml \approx 10 g tissue) into a r.b. flask and rotary evaporate down to 20 mls to remove methanol.
- (d) Place the aqueous extract onto an Extrelut partition column and allow it to percolate onto the column for exactly 15 minutes. Elute the Extrelut column with hexane (50 ml) and dichloromethane (50 ml) and collect both organic eluates into a single 250 ml r.b. flask. (This fraction contains the parent permethrin extracted and any "free" 3-phenoxybenzyl alcohol).
- (e) Wash the Extrelut column with methanol (100 ml) and collect this eluate in a 250 ml r.b. flask. Evaporate the extract down to 20 ml aqueous residue (this fraction contains all acidic metabolites and any conjugated 3-phenoxybenzyl alcohol).

5.3.2 Milk

- (a) Measure a well mixed sample of milk (10 ml) into a 500 ml Mason jar and make basic by the addition of 0.1N sodium hydroxide solution (10 ml).
- (b) Add 50% v/v acetone : hexane (80 ml) and blend at medium speed using a Sorvall macerator for 5 minutes.
- (c) Transfer the mixture to a 250 ml separating funnel and allow the phases to separate.

Run-off the lower, aqueous, phase containing the acidic metabolites and conjugated 3-PB alcohol, into a r.b. flask. Collect the remaining organic phase, containing parent permethrin and any free 3-PB alcohol in a further r.b. flask.

5.4

Hydrolysis and Partition of Metabolites

- (a) Add concentrated hydrochloric acid (2.2 ml) to the aqueous fractions (20 ml) obtained from 5.3.1 (e) and 5.3.2 (c) and heat under reflux for 2 hours to hydrolyse any conjugated metabolite residues.
- (b) On cooling, add ammonium chloride (0.5g) and transfer the hydrolysed aqueous extract onto an Extrelut partition column. Allow the extract to percolate into the Extrelut matrix for 15 minutes.
- (c) Elute the Extrelut column with diethyl ether (50 ml) followed by dichloromethane (50 ml) and collect the combined organic eluate in a r.b. flask.
- (d) Evaporate the mixture down to small volume and redissolve the extracts in dichloromethane (50 ml).
- (e) Transfer the dichloromethane to a 100 ml sep. funnel and partition with 1M sodium hydroxide solution (2 x 25 ml). Collect the basic fraction (containing all the acidic metabolites) and the dichloromethane fraction (containing liberated 3-PB alcohol residues) separately.
- (f) Combine the dichloromethane fraction with the organic extracts of "free" 3-PB alcohol obtained in 5.3.1(d) or 5.3.2(c) and set aside for derivatisation to the heptafluorobutyryl 3-PB alcohol.
- (g) Acidify the basic extracts (from (e) above) to $pH < 2$ by the addition of concentrated hydrochloric acid. Partition the acid phase with diethyl ether (25 ml) followed by dichloromethane (25 ml).
- (h) Combine the two organic extracts into a r.b. flask and evaporate down to a small volume, 2ml, using a rotary evaporator.

- (i) Transfer the small volume remaining in the r.b. flask to a 10 ml graduated centrifuge tube and evaporate to dryness using a gentle stream of dry air. Thoroughly rinse the r.b. flask with further dichloromethane (2 x 2 ml) and transfer each rinse to the centrifuge tube, blowing to dryness as above on each occasion (this procedure removes traces of water azeotropically). Set aside for methylation of the acidic metabolites using diazomethane.

5.5 Derivatisation of 3-Phenoxybenzyl Alcohol Residues
using Heptafluorobutyric Anhydride

- (a) Evaporate the combined organic extracts containing 3-PB alcohol residues (5.3.1(d) or 5.3.2(c) and 5.4(f)) almost to dryness using a rotary evaporator. Transfer the small volume remaining to a 10 ml graduated centrifuge tube and evaporate to dryness using a gentle stream of dry air. Thoroughly rinse the r.b. flask with further dichloromethane (2 x 2 ml) and transfer the rinse to the centrifuge tube, evaporating to dryness as above on each occasion to azeotropically remove water.
- (b) Add 0.4% v/v pyridine : chloroform (1 ml) followed by heptafluorobutyric anhydride (100 μ l). Tilt the centrifuge tube to thoroughly wash down the walls of the tube, stopper, and allow to stand at room temperature for 15 minutes.
- (c) Evaporate off excess reagent and solvent under a gentle stream of dry air (N.B. in a fume cupboard) and add toluene (5 ml). Thoroughly mix the contents of the tube and add 3% w/v sodium bicarbonate solution (3 ml).
- (d) Mix the tube and contents for 15 seconds. Remove an aliquot of the toluene (2 ml) and transfer to the top of a hexane equilibrated Florisil (1.5 g) column capped with granular anhydrous sodium sulphate (0.5 g). Elute the column with toluene (10 ml) and collect the eluate in a graduated centrifuge tube.
- (e) Evaporate the toluene to exactly 4 ml under a gentle stream of dry air to give a final sample to solvent ratio of 1.0g ml⁻¹. (1.0ml ml⁻¹ for milk samples).

- (f) Calibration analytical standards are prepared by derivatising accurately known amounts (usually 1 - 10 µg) of 3-PS alcohol by the procedure described to give final solutions containing 0.1 - 1.0 µg ml⁻¹ of 3-PS alcohol equivalents.

5.6 Methylation of Acid Metabolite Residues using Diazomethane

N.B. All operations involving diazomethane must be carried out in a fume cupboard.

- (a) Add methanol (0.5 ml) to the dried extract containing the acid metabolites obtained in 5.4(i) and thoroughly rinse down the walls of the tube by tilting the centrifuge tube. Add freshly prepared ethereal diazomethane (1 ml) and plug the end of the tube with cotton wool. Ultrasonicate the mixture for 30 minutes.
- (b) Evaporate the excess methylating reagent down to 0.5 ml under a gentle stream of dry air. Add hexane (5.0 ml) and water (4.5 ml) and shake for 15 seconds. Allow the phases to separate.
- (c) Remove an aliquot of the upper hexane layer (4.0 ml) and transfer to a second centrifuge tube. Concentrate the solvent to exactly 0.8 ml i.e., to give a sample to solvent ratio of 10 g ml⁻¹ (10 ml ml⁻¹ for milk samples).
- (d) Standard solutions containing accurately known amounts of each of the acid metabolites (usually 0.1 - 1.0 µg) are prepared by the procedures described to give standard solutions containing 1.0 - 10.0 µg ml⁻¹ of each acid equivalent.

6. CONTROL AND RECOVERY EXPERIMENTS

- (a) Untreated control samples must be analysed using the procedures described in order to demonstrate that endogenous substances present in the samples do not interfere with the final determination of any of the metabolite derivatives.
- (b) Recovery experiments in which untreated control samples are accurately fortified with a known amount of each metabolite must be analysed alongside any batch of samples in order to determine the percentage efficiency of recovery through the procedures used. The % recovery of 3-PS alcohol should be examined both by fortification of the original sample prior to extraction and also by fortification of the sample after the hydrolysis step.

Summary of % recovery data obtained when control cow tissues and milk were accurately fortified prior to extraction.

Tissue	DC7 mono acid	3-PB alcohol	3-PB acid
liver	71,82	83,81	46,70
kidney	72,63	94,80	55,89
pectoral muscle	56,92	88,80	52,49
adductor muscle	64,55	98	65,63
cardiac muscle	66,60	77,92	67,70
milk	62,57	50,58	64,53
mean recovery (tissue)	69%	84%	63%
standard deviation	11%	6%	13%

7. FINAL QUANTITATIVE DETERMINATION OF METABOLITE RESIDUES

7.1 Gas Liquid Chromatographic Determination of 3-PB Alcohol as the Heptafluorobutyryl Derivative

- (a) Make repeated injections of 2-5 µl of an analytical standard solution, e.g. 0.1 µg ml⁻¹ of 3-PB alcohol as the heptafluorobutyryl derivative, prepared as described in 5.5(f), into an EC-GLC operated under the conditions given in 5.1. When a consistent response is obtained measure the peak height (or peak area when electronic integration facility is available) recorded for the standard solution.
- (b) Inject 2-5 µl of the derivatised sample solution, obtained from 5.5(e), and similarly measure the response at the retention time of the 3-PB alcohol HFB derivative.
- (c) Calculate the measured residue, expressed in mg kg⁻¹ (for tissue samples), or mg L⁻¹ (for milk samples) using a simple proportionation calculation i.e.

$$\frac{R_S}{R_A} \times \frac{V_A}{V_S} \times \frac{C_A}{C_S} = \text{measured residue (mg kg}^{-1} \text{ or mg L}^{-1}\text{)}$$

when :

R_S = Response (peak height or area) of sample.

R_A = Response (peak height or area) of analytical standard solution).

V_S = Volume (µl) of sample injected.

V_A = Volume (µl) of analytical standard injected.

C_S = Concentration (g ml⁻¹) tissue, (ml ml⁻¹) milk, of final sample solution.

C_A = Concentration (µg ml⁻¹) of analytical standard solution.

- (d) Correct the measured residue value calculated above for the mean % recovery obtained for the recovery experiments carried out simultaneously with the samples e.g. for a mean 75% recovery corrected residue = measured residue $\times \frac{100}{75}$.

7.2

GCMS - Selected Ion Monitoring Determination of DCV Monocacid and 3-PB Acid as their Methyl Esters

- (a) Inject 2-5 μl of a strong standard solution, e.g. 100 $\mu\text{g ml}^{-1}$, of a derivatised acid metabolite into the GCMS operated under the conditions described in 5.2.
- (b) Tune the mass spectrometer in order that a maximum response is obtained when the standard solution is repeatedly injected, i.e. ensure that the SIM device is set precisely at the required m/z values.
- (c) Increase the amplification gain in order that the sensitivity is sufficient to give a measurable response at the limit of determination for each m/z recorded. Inject 2-5 μl of a control sample and observe the response on each m/z value recorded at the retention time of the acid metabolite methyl ester. Select the m/z channel which will have the highest signal to noise ratio for residues of the metabolite and use this for quantitative measurements.
- (d) Select an appropriate analytical standard (prepared 5.6(d)) e.g. 1.0 $\mu\text{g ml}^{-1}$ and make repeated injections until a constant response is obtained for the respective m/z values for the metabolites). Measure the peak height recorded on the relevant m/z channel for each metabolite.
- (e) Inject 2-5 μl of the sample solution (5.6(c)) and similarly measure the response as peak height recorded at the respective retention times of each metabolite.
- (f) Calculate the measured residue in mg kg^{-1} for tissue samples or mg L^{-1} for milk samples by proportionation as described previously (7.1(c)).

- (g) Calculate the corrected residue values by normalising for mean \pm recovery for the appropriate metabolite in a similar manner to that described 7.1(d).

8.

EXAMPLES OF FINAL CHROMATOGRAPHIC TRACES

- Fig 2 - Electron capture GLC trace for the final determination of 3-PP alcohol as the heptafluorobutyryl derivative in liver extract samples.
- Fig 3 - Selected ion monitoring (m/z 187) GCMS trace obtained for the analysis of DCV monoacid as the methyl ester derivative in liver extract samples.
- Fig 4 - Selected ion monitoring (m/z 229) GCMS trace obtained for the analysis of 3-PP acid as the methyl ester derivative in liver extract samples.

FIGURE 2

Electron capture GLC trace for the final determination of 3-PB alcohol as the heptafluorobutyryl derivative in liver extract samples.

1.0 g ml⁻¹
Liver extract
of cow no. 37
corrected
residue
= <0.01 mg kg⁻¹

1.0 g ml⁻¹
Liver extract
of cow no. 25
corrected
residue
= 0.10 mg kg⁻¹

0.1 ug ml⁻¹
Standard
solution

3-PBA HFB

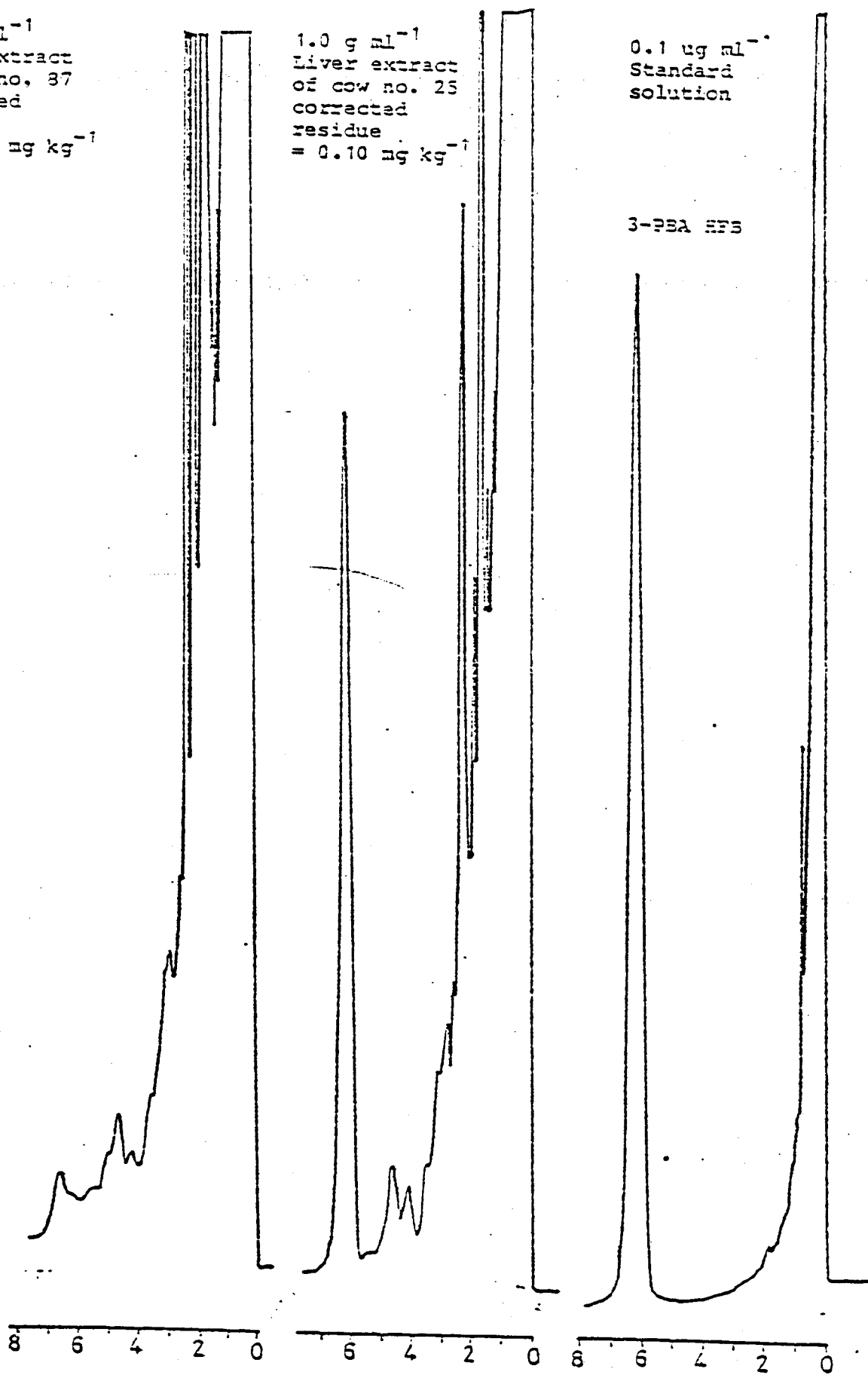


FIGURE 3

Selected ion monitoring (m/z 167) GCMS trace for the final determination of DCV mono acid as the methyl ester in liver extract samples.

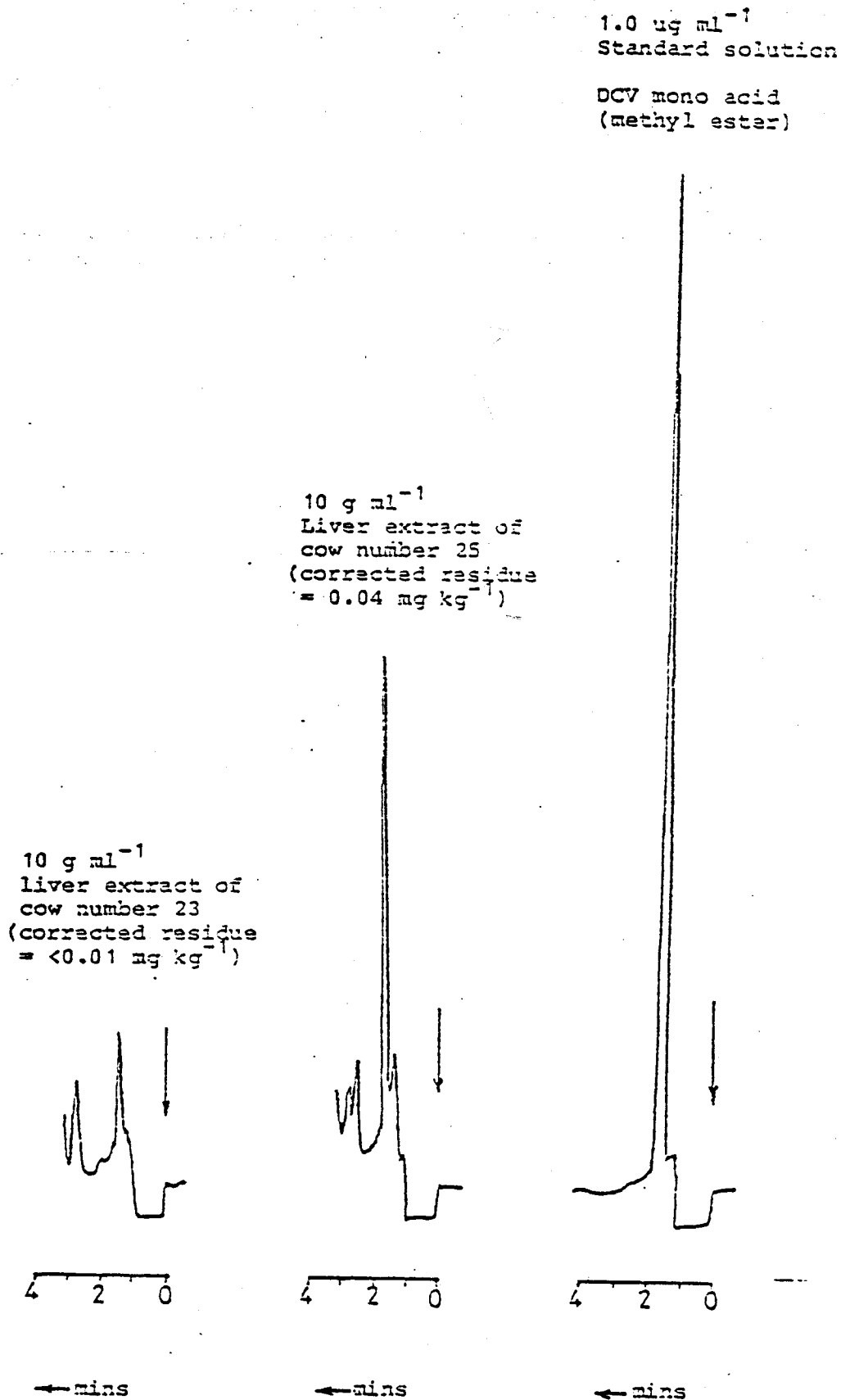
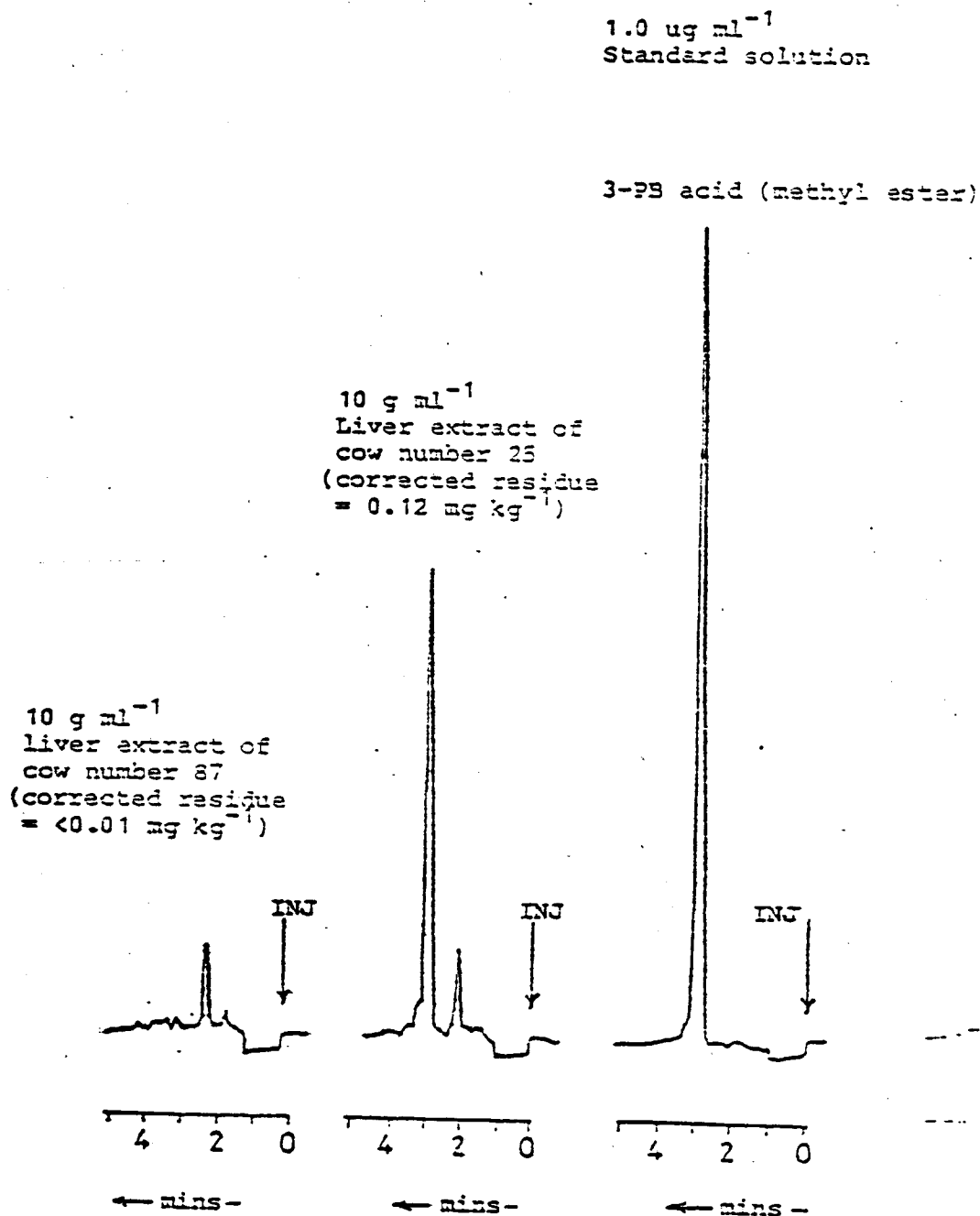


FIGURE 4

Selected ion monitoring trace (m/z 228) GCMS trace for the final determination of 3-PS acid as the methyl ester in liver extract samples.



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Date 1/24/79

From T. R. Nelsen

cc

Subject ANALYTICAL REPORT

M-4329

Project No. and Title: G138-Pyrethroid Insecticide-FMC 33297

Author: T. R. Nelsen

Supervisory Personnel: R. F. Cook

Work Done By: J. E. Burt, M. H. Gruenauer, G. E. Lover and T. R. Nelsen

Period Covered: December 15, 1978 - January 20, 1979

References: C8747, 74-94

Previous Report: W-0232

Title: DETERMINATION OF DICHLOROVINYL ACID RESIDUES IN/ON
SOYBEAN PROCESSING PRODUCTS

ABSTRACT

Samples from a soybean processing study were analyzed for residues of cis and trans dichlorovinyl acid (DCVA). The processed beans had been treated with Pounce® 3.2 EC at 0.11 kg ai/ha (0.1 lb ai/A) in a two application foliar spray program. The mature beans were harvested 21 days following the second application. No detectable DCVA residue was found in/on the treated soybean, solvent extracted meal or refined oil. DCVA residues estimated to be 0.02 µg/g (ppm) were found in/on the soybean hulls.

The method of analysis involved a methanol/water blend, acid hydrolysis, hexane partition, butylation and florisil column cleanup. Detection of DCVA as its butyl ester was accomplished by electron capture (⁶³Ni) gas chromatography. Method sensitivity was 0.05 µg/g (ppm) and method detectability was 0.01 µg/g (ppm).

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I. INTRODUCTION

Permethrin formulated as an emulsifiable concentrate, Pounce® 3.2 EC, is currently being developed as a foliar insecticide on soybeans. Previous studies have determined permethrin residue levels in/on soybeans (A1, A2, A3) resulting from treatment with Pounce® 3.2 EC. A study reporting residue levels of dichlorovinyl acid, DCVA (FMC 30062), a known plant metabolite of permethrin resulting from a field spray program has also been issued (A4). A report concerning parent (permethrin) residue levels found in/on soybean processing products has also been issued (A5).

The data reported herein covers the DCVA residue levels found in/on subsamples of the same processing parts, from the study cited above (A5). In addition, report M-4328 (A6) presents the meta-phenoxybenzyl alcohol (MPBA) residue data for this same processing study. MPBA is also a known major plant metabolite of permethrin.

II. SAMPLE HISTORY

Soybeans (variety Tracy) were grown in a test plot in James, Mississippi. The plants were treated with Pounce® 3.2 EC at 0.11 kg ai/ha (0.1 lb ai/A) in a two application foliar spray program. Treatments were applied by aircraft and 1.0 gallon per acre of finished spray was used per application. The first treatment was applied at early pod development and the second 21 days before harvest. At maturity, the soybeans were harvested and a representative sample was shipped to the Oilseed Products Division at Texas A&M University for processing.

The soybeans were cracked and hulls removed from meats. The meats were flaked to 0.015-0.020 inches in thickness and solvent extracted using Skellysolve F. Crude oil was recovered from the solvent. The crude oil was refined using the official laboratory method of the American Oil Chemist Society (B1). The refined oil was bleached and deodorized. Samples of unprocessed beans, hulls, solvent extracted meal, refined oil and alkaline soapstock were collected and shipped to the FMC residue analysis lab at Richmond, California, where analysis for permethrin were performed. A subsample of each part was sent to the FMC residue analysis laboratory at Middleport, New York. The samples were stored at -20°C until analyzed.

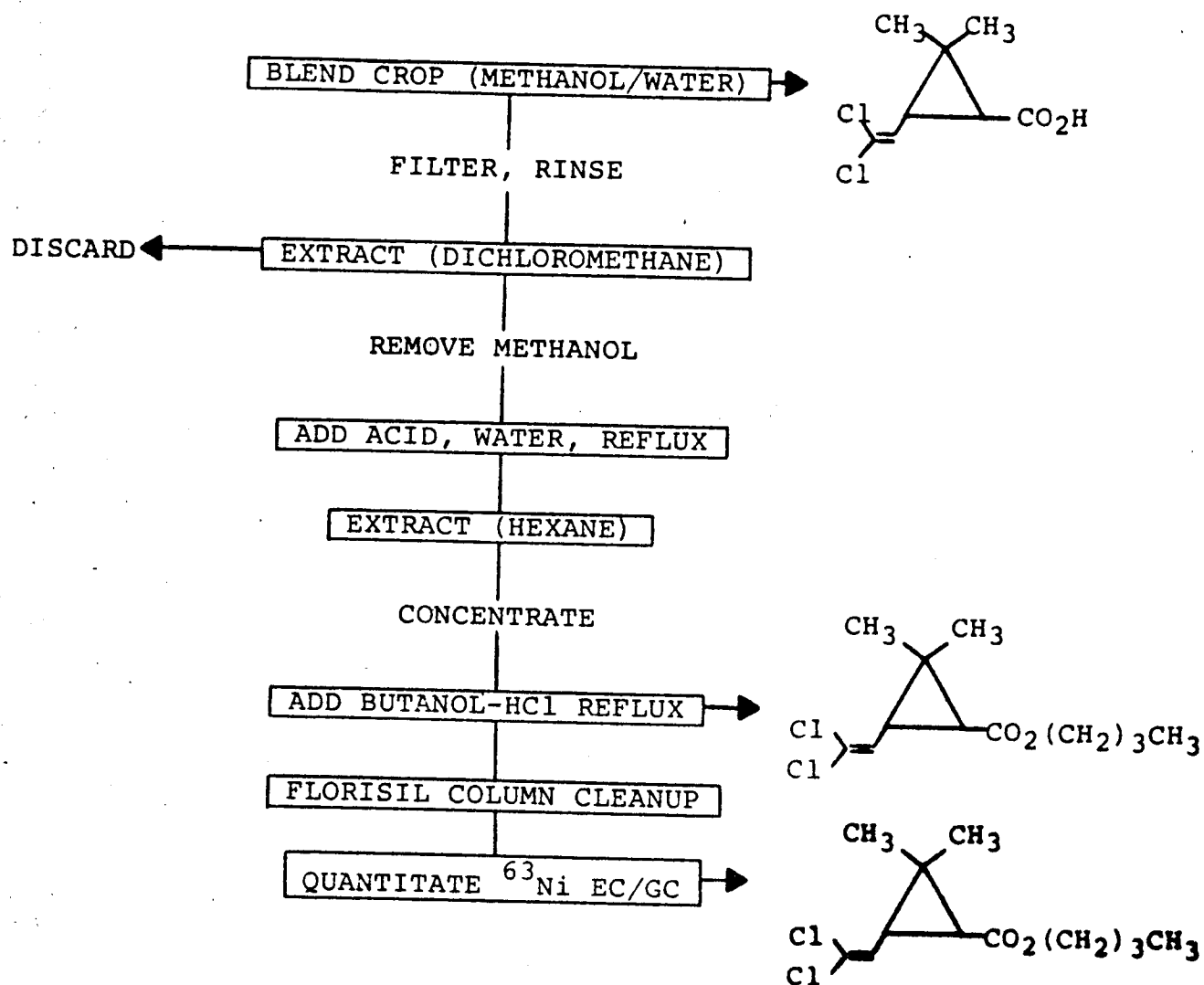
III. ANALYTICAL METHOD

Figure 1 diagrams the method of analysis employed for the DCVA residue determination.

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FIGURE 1

FLOW SCHEME FOR THE ANALYSIS OF DCVA IN/ON SOYBEANS



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Apparatus

Condensor, Reflux
Food Chopper, Hobart Model 4114 (or equivalent)
Food Blender, Waring
Flask, round bottom 250 ml with 24/40 $\frac{1}{2}$ fittings
Flask, Vacuum Filter 500 ml
Flask, round bottom 100 ml
Funnel, Buchner glass with coarse sintered glass frit
Funnel, separatory 250, 500 ml
Liquid chromatographic column, 19 mm I.D. X 15 cm with
200 ml reservoir
Kuderna-Danish evaporative concentrator
Synder column, 280 mm
Steam bath
Assorted standard laboratory glassware

Instrumentation

Gas chromatograph - Hewlett-Packard 5700 equipped with
a ^{63}Ni electron capture detector (ECD)
Column gas chromatographic, 2 mm X 122 cm packed with
5% OV-210 on 80-100 M chromosorb WHP (Supelco)

Reagents

Argon/methane (95/5) compressed gas
1-Butanol distilled in glass (Burdick & Jackson)
Celite 545, Fisher
Chemsolve[®], Mallinckrodt
Dichloromethane, laboratory distilled
Ethyl acetate, distilled in glass, Burdick & Jackson
Florisil, 100/200 m, Floridin Co.
Glass wool, fiber, Pyrex
Hexane, resi-analyzed, Baker
Hydrochloric acid, concentrated
Hydrogen chloride, compressed gas

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Procedure (Hulls, Solvent Extracted Meal)

All glassware was thoroughly washed with a non-phosphorous detergent, water rinse, Chemsolve (or caustic) soak, water rinse, and final methanol rinse.

Twenty grams of macerated crop was blended with 200 ml 2:1 methanol/water (v/v) at high speed for five minutes and filtered through a bed of Celite. The filter pad was rinsed with an additional 50 ml of blending solution. The blend solution was made basic (pH \approx 8.3) by the drop-wise addition of 0.1N NaOH. The solution was extracted twice with 250 ml dichloromethane (DCM). The DCM was discarded. One half the sample by volume (equivalent to ten grams crop) was concentrated to 10-30 ml on a Rinco[®] vacuum concentrator. Water and concentrated hydrochloric acid were added to give 100 ml at 1.0N. This aqueous solution was refluxed 1 hour and cooled. The hydrolysate was extracted twice with 100 ml hexane. The hexane was dried over a minimum amount of sodium sulfate which was prerinsed with ethyl acetate. The hexane was concentrated on a steam bath in a Kuderna-Danish concentrator to @ 5 ml. The residue was taken just to dryness under a gentle stream of nitrogen. To this was added 10 ml of butanol which was saturated with gaseous hydrogen chloride. The mixture was refluxed 0.5 hours employing a water trap to control HCl fumes and cooled. The condensers were rinsed with two 10 ml portions distilled water. This mixture was transferred to a separatory funnel. Five ml 6N sodium hydroxide was added and the mixture extracted 3 times with 40 ml hexane. The hexane extract was washed with 120 ml 0.1N sodium hydroxide. The hexane was dried with a minimum amount (1-2 g) of prerinsed sodium sulfate. The mixture was concentrated in a Kuderna-Danish concentrator to 5 ml and reduced further to 1 ml under a gentle stream of nitrogen. This material was then subjected to an open column cleanup. The column was prepared in the following manner.

A 150 mm X 19 mm (i.d.) glass column equipped with a teflon stopcock and 250 ml bulb reservoir was used. A plug of glasswool (prerinsed with ethyl acetate) and 10 g of florisil (100-200 M) which was deactivated to a moisture content of 2.8 ± 0.3 weight percent and 0.5 g anhydrous sodium sulfate (prerinsed with ethyl acetate) were placed sequentially in the column. The column was prewet with 20 ml hexane. The sample was transferred to the column with several 1 ml rinses of hexane. Fifty ml of hexane were eluted and discarded. Fifty ml 10% ethyl acetate/hexane (v/v) were then eluted and collected. Three ml toluene were added and the eluent was reduced

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to @ 5 ml on a steambath in a Kuderna-Danish concentrator. This residue was transferred to a graduated centrifuge tube. The sample was concentrated under a gentle stream of nitrogen to exactly 3 ml for quantitation.

Procedure (Oil)

The procedure for oil was the same as that for hulls and meal except that the initial methanol/water blend was extracted twice with 100 ml DCM and twice with 100 ml hexane and these two organic phases discarded.

Procedure (Soybean)

The procedure for soybeans was the same as that for hulls and meal except a second cleanup column was employed. The eluent from the 10 g florisil column was concentrated to 1 ml. This residue was transferred to a 10 mm (i.d.) X 120 mm column containing 1.0 g of activated (24 hours at 120°C, moisture content @ < 0.5%) florisil. The column was capped with 1.0 g prerinsed sodium sulfate. The column was eluted with 15 ml toluene. The toluene eluent was then reduced to exactly 3 ml under a gentle stream of nitrogen for quantitation.

Analysis

Detection of the butyl esters of cis and trans DCVA was accomplished using a Hewlett-Packard 5730 gas chromatograph equipped with a ^{63}Ni electron capture detector.

Detector Parameters -

Temperature: 350°C
Attenuation: 5

Column Parameters -

Length: 122 cm
Diameter: 2 mm (i.d.)
Packing: 5% OV-210 on 80-100M Chromosorb WHP (Supelco)
Injector Temperature: 200°
Column Temperature: 130-140° as required for resolution
Carrier: 95/5 Argon/Methane at 50 ml/min
Recorder: HP 3380A attenuation 32

Retention times of cis- and trans-DCVA butyl esters varied from 5.5-7.7 (cis) and 6.9-9.7 (trans) as a result of the difference in column temperature required for analysis resolution.

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V. QUANTITATION

Quantitation of residue values were made employing the following formula:

$$\text{ppm} = \frac{\text{peak height unknown} \times \text{ng standard}}{\text{peak height standard} \times \text{mg crop injected}} \times \text{CF}$$

Where CF = 0.787, the molecular weight ratio of DCVA to DCVA butyl ester.

The following is an example calculation. The standard peak height and fortified height numbers are from the chromatograms shown in Figure 2.

Peak Height 2.12 ng trans butyl DCVA = 23 mm

Peak Height Sample = 10 mm (trans)

Crop Injected = 6.67 mg

$$\text{ppm } (\text{trans}) = \frac{10 \times 2.12}{23 \times 6.67} \times 0.787 = 0.109$$

Standard Preparation

The following standards were used for fortifications and gas chromatographic analysis:

<u>Compound</u>	<u>Reference Number</u>
DCVA (27.8% <u>cis</u> , 72.2% <u>trans</u>)	C8747-6
DCVA (100% <u>trans</u>)	C8747-6
DCVA Butyl Ester (100% <u>cis</u>)	C7439-52-1
DCVA Butyl Ester (100% <u>trans</u>)	C7439-52-2

Standard Stock Solutions

One hundred mg of solid or as close to 100 mg as possible liquid standards were accurately weighed into 100 ml volumetric flasks. These solutions were made to volume with 100 ml toluene resulting in 1 µg/ul stock solutions. These solutions were stored at 0°C in the dark.

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Fortification Standard Solution Preparation

Exactly 1 ml of each of the dichlorovinyl acid stock solutions were diluted to 10 ml with hexane. This resulted in 0.1 µg/µl fortification solutions.

Standard Injection Solution

Ten µl of each the cis and trans DCVA-butyl ester stock solutions were diluted in 10 ml volumetric flasks with toluene. This resulted in a standard solution which contained 1.05 ng/µl cis and 1.06 ng/µl trans-DCVA-butyl ester, respectively.

Routinely, 2 to 3 µl out of 3,000 µl of the butylated extract (equivalent to 6.67 to 10 mg of crop) was injected for quantitation. The choice of injection volume was dependent on the detector sensitivity at that point in time.

ANALYTICAL LIMITS

Quantitatively reliable measurement of response (i.e., method sensitivity) was determined and validated by satisfactory recovery data from fortified check samples. Method sensitivity was determined to be 0.05 µg/g (ppm) for both the cis and trans isomer of DCVA. Visual recognition of detector response as a peak was possible when the detector response exceeded 1 mm in height (0.005 µg/g). Responses up to 2 mm were considered indistinguishable from average instrument operating background and defined as the limit of method detectability (0.01 µg/g). Responses below the method detectability (0.01 µg/g) are reported as non-detectable (ND). Detector responses greater than method detectability (0.01 µg/g) and less than method sensitivity (0.05 µg/g) are reported as less than the method sensitivity (< 0.05 µg/g). Any value reported in this area (0.01-0.05 µg/g) must be considered to be an estimated value, as the quantitative reliability of a number in this region is highly questionable.

The method sensitivity is limited by coextractives, particularly in the area of cis-DCVA butyl ester. The size of this response relative to responses < 0.05 would make quantitation below this level subject to significant analytical variation.

FORTIFICATION RECOVERIES

Soybean processing product check samples were fortified prior to the addition of the blending solution (2:1, methanol/water, v/v). The fortification standard (dissolved in hexane) was added with a microliter syringe onto the crop and the solvent allowed to evaporate for 5 to 10 minutes before the addition of the blending solution.

The DCVA fortification solutions were prepared by taking 1000 μ l (1 ml) of the standard stock solutions (quantitation section, page 5) and diluting to 10 ml with hexane. This resulted in a 0.1 μ g/ μ l solution. Check samples (10 g) were fortified with 5 to 36 μ l (0.5-3.6 μ g) of the fortification solutions. Specific data for recovery experiments are found in Table 1.

TABLE 1

RECOVERY OF CIS AND TRANS DCVA FROM FORTIFIED SOYBEAN PROCESSING PARTS

CHECK SAMPLE IDENTIFICATION	MATRIX	FORTIFICATION LEVEL (PPM)		RECOVERY LEVEL (PPM)		RECOVERY (%)	
		CIS	TRANS	CIS	TRANS	CIS	TRANS
SBP-2164-78	Soybean	0.05	0.13	.036	.109	72	83
SBP-2164-78	Soybean	0.10	0.26	.086	.226	86	87
SBP-2164-78	Soybean	NF	0.05	NF	.035	NF	70
				Average		79	80%
SBP-2168-78	Soybean Hull	0.05	0.13	.040	.093	80	72
SBP-2168-78	Soybean Hull	NF	0.05	NF	.043	NF	86
				Average		80	79%
SBP-2166-78	Solvent	0.05	0.13	.031	.080	62	62
	Extracted Meal						
SBP-2166-78	Solvent	0.10	0.26	.062	.159	62	61
	Extracted Meal						
SBP-2166-78	Solvent	NF	0.05	NF	.036	NF	72
	Extracted Meal						
				Average		62	65%
SBP-2172-78	Refined Oil	0.05	0.13	.029	.059	58	45
SBP-2172-78	Refined Oil	0.10	0.26	.058	.154	58	59
SBP-2172-78	Refined Oil	NF	0.05	NF	.032	NF	64
				Average		58	56%

NF means not fortified

III. RESULTS

Results from individual analysis are summarized on Table 2 (pg 13).

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VII. RESULTS (Continued)

Typical chromatograms are shown in Figures 2-5 (pg 14-17). The ppm values shown on the chromatograms are uncorrected.

VIII. DISCUSSION

No detectable residue of DCVA were found in/on any soybean processing product analyzed. Low level DCVA residue, estimated to be 0.02 µg/g (ppm) was found in/on soybean hulls.

IX. CONCLUSION

No significant residue of DCVA exist in/on permethrin treated soybean products after processing. Further, no concentration of non-detectable bean residues into the primary processing products (i.e., meal and oil) occurred.

X. LITERATURE CITED

A. FMC Literature

1. G. H. Fujie, "Determination of Parent FMC 33297 in/on Soybeans", FMC Corporation, Agricultural Chemical Group, Richmond, CA, report W-0123 (1976).
2. G. H. Fujie, "Determination of Parent FMC 33297 in/on Soybeans", FMC Corporation, Agricultural Chemical Group, Richmond, CA, report W-0209 (1977).
3. G. H. Fujie, "Determination of Parent FMC 33297 in/on Soybeans", FMC Corporation, Agricultural Chemical Group, Richmond, CA, report W-0231 (1978).
4. T. R. Nelsen, "Determination of Dichlorovinyl Acid Residues (FMC 30062) in/on Soybeans", FMC Corporation Agricultural Chemical Group, Middleport, NY, report M-4282 (1978).
5. G. H. Fujie, "Determination of Permethrin Residue Levels in/on Soybean Processing Products from a Soybean Processing Study", FMC Corporation, Agricultural Chemical Group, Richmond, CA, report W-0232 (1978).
6. T. R. Nelsen, "Determination of Meta-Phenoxybenzyl Alcohol Residues in/on Products from a Soybean Processing Study", FMC Corporation, Agricultural Chemical Group, Middleport, NY, report M-4328 (1979).

B. Published Literature

1. "Official Laboratory Method", American Oil Chemist Society, Ca 9b-5a.

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AUTHOR:

P.R. Nelson

DATE:

2/20/79

READ & UNDERSTOOD BY:

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DATE:

20 FEB 79

TRN/cmf

Attachments

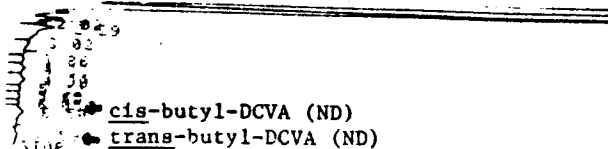
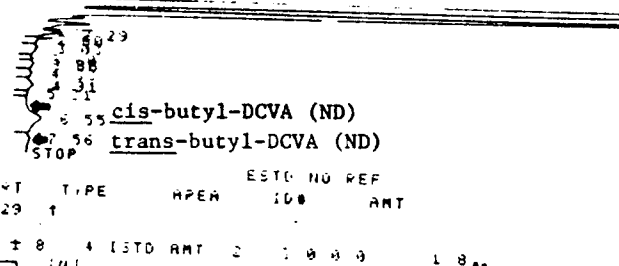
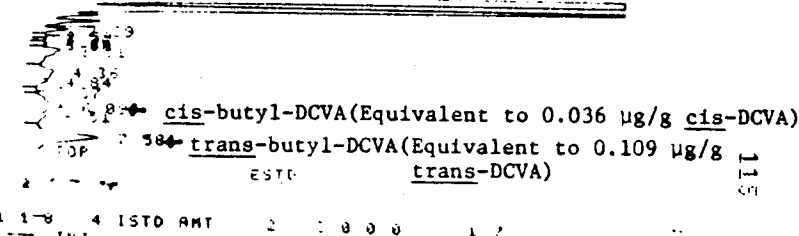
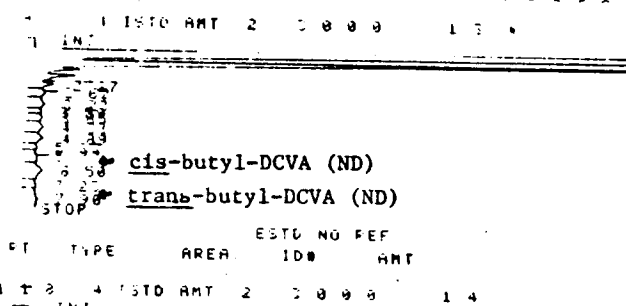
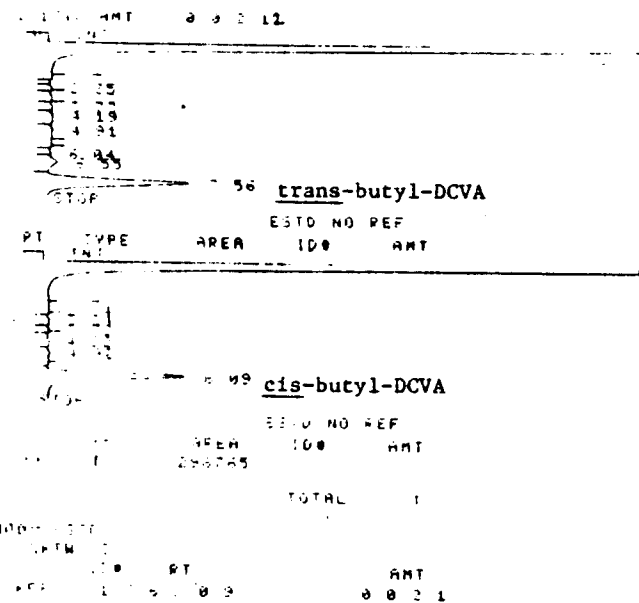
Date Submitted for Typing: January 23, 1979Date Submitted for Corrections: January 25, 1979Date Submitted for Issue: February 22, 1979

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Standard Injection
2.1 ng cls-
butyl-DCVA

Soybean Check
Sample
SBP-2164-78
6.67 mg infected

Soybean Check	Soybean Treated	Soybean Treated
Sample	Sample	Sample
SBP-2164-78	SBP-2165-78	SBP-2165-78
Fortified with	6.67 mg Injected	6.67 mg
0.05 $\mu\text{g/g}$ <u>cis</u> -		Injected
DCVA + 0.13 $\mu\text{g/g}$		
<u>trans</u> -DCVA		
6.67 mg Injected		



TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF CIS AND TRANS DCVA IN SOYBEANS

FIGURE 2

Soybean Hull
Treated Sample
SBP-2169-78
6.67 mg Injected

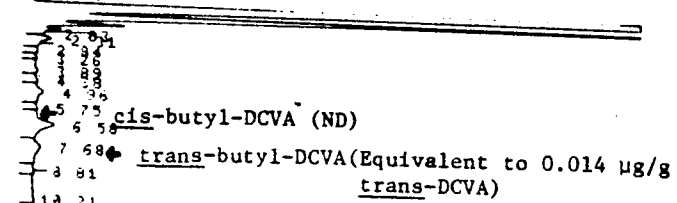
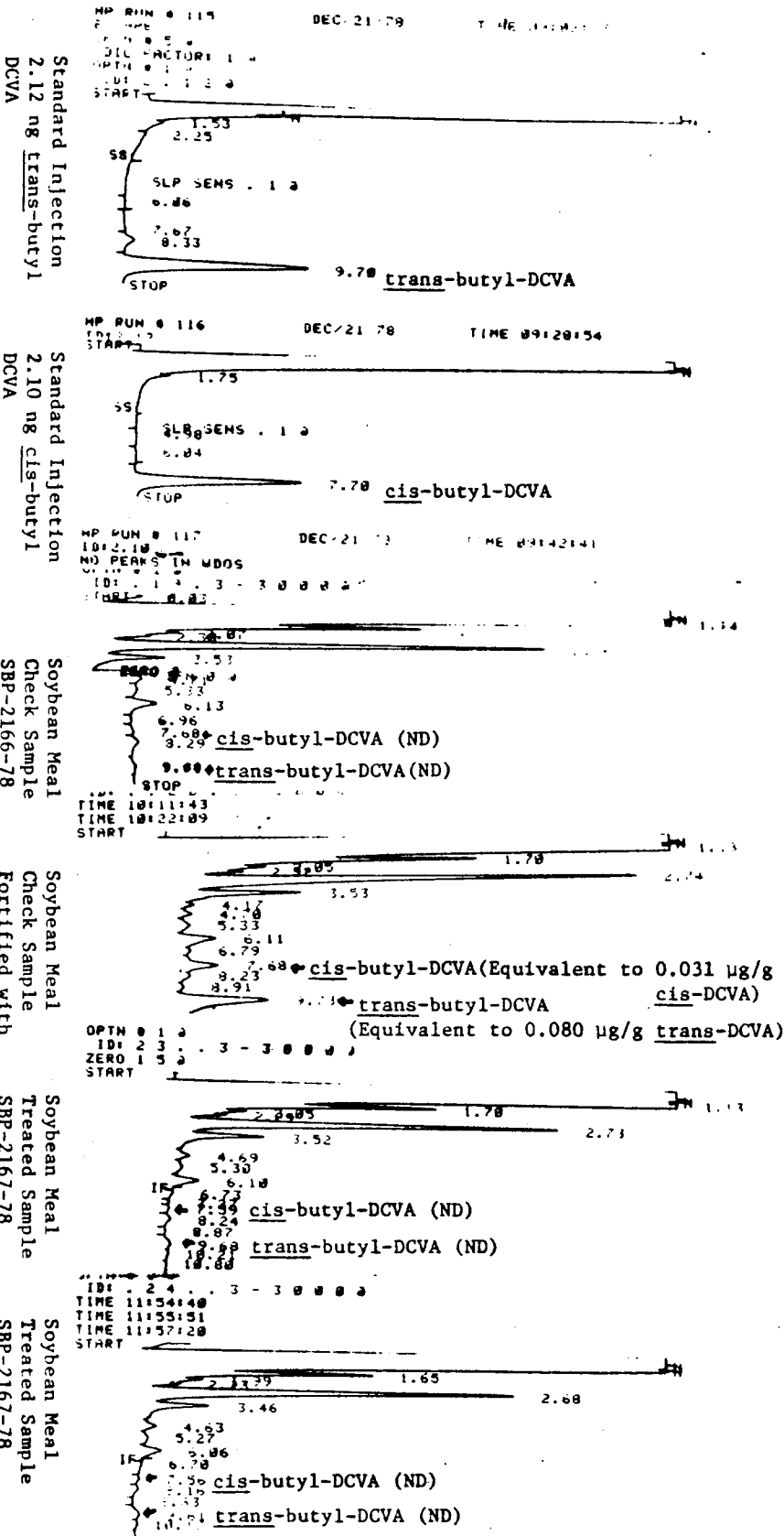


FIGURE 3

TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF CIS AND TRANS DCVA IN SOYBEAN HULLS

TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF CIS AND TRANS DCVA IN SOLVENT EXTRACTED SOYBEAN MEAL

FIGURE 4



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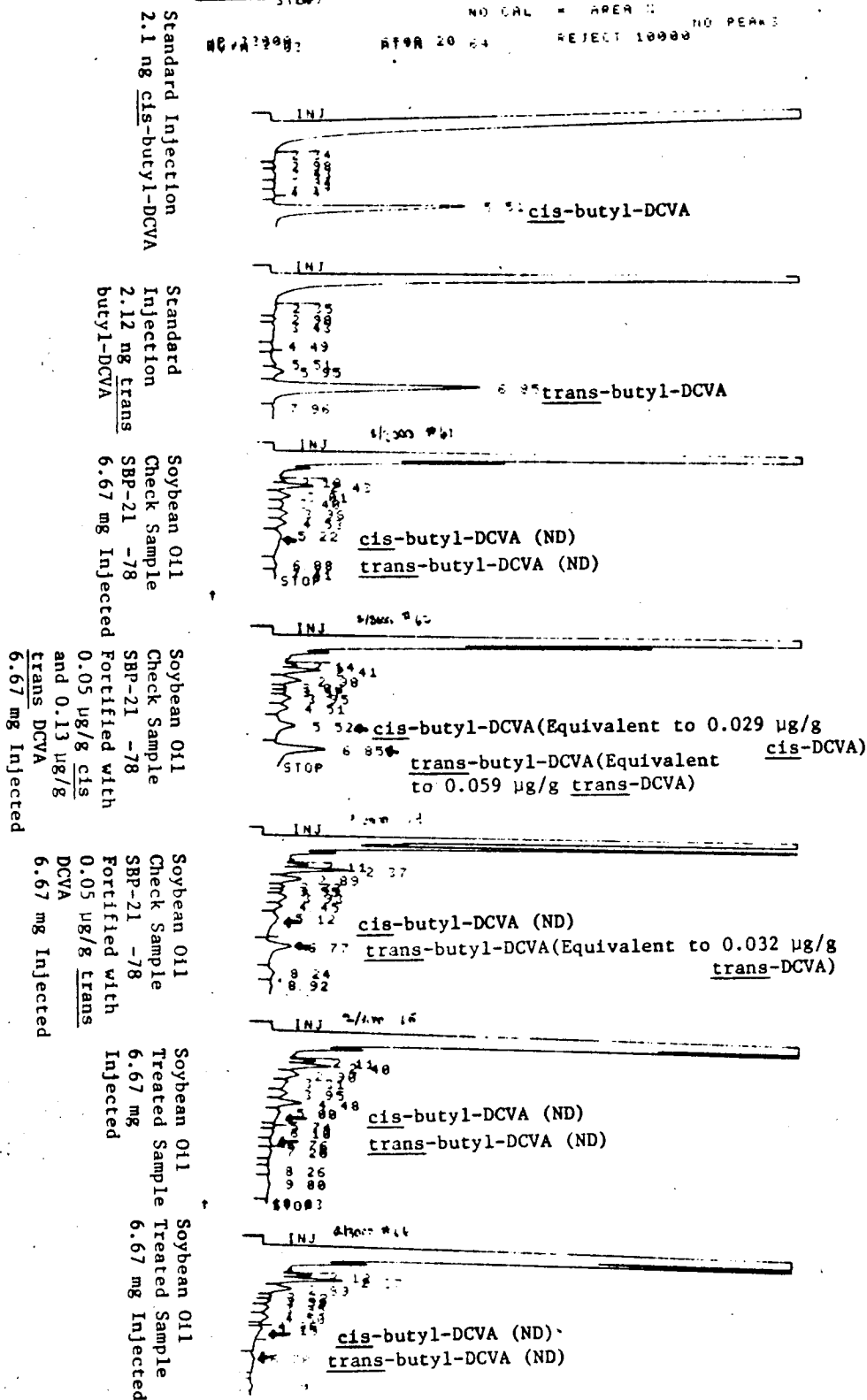


FIGURE 5
TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF CIS AND TRANS DCVA IN REFINED BLEACHED DEODORIZED SOYBEAN OIL

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